CaO-SiO_2-BASED BIOACTIVE GLASS AND SINTERED CALCIUM PHOSPHATE GLASS USING SAME

FIELD OF THE INVENTION

The present invention relates to a CaO-SiO_2-based bioactive glass usable in bone restoration materials such as artificial joints, artificial dental roots and artificial bones, and a sintered calcium phosphate glass using the bioactive glass.

BACKGROUND OF THE INVENTION

When an artificial material is implanted in a damaged region of a living body, the material is generally surrounded by membranes of collagen fibers and thus isolated from neighboring bones. However, there have been known some artificial materials, which are not isolated by such fibrous membranes and strongly connect to bones in a living body. Examples of such artificial materials include Na_2O-CaO-SiO_2-P_2O_5-based bioglasses, sintered hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, and crystallized glasses. Known as the crystallized glasses are, for example, CaO-MgO-SiO_2-P_2O_5-based bioactive glasses containing wollastonite crystals and apatite crystals such as hydroxyapatite crystals. These materials are referred to as bioactive ceramics, and some of them have put into practical use as important bone restoration materials.

The sintered hydroxyapatites have been widely used in medical treatments as bone restoration materials with high biocompatibility, and production methods thereof have been widely studied. With demand for more biocompatible artificial bones, etc. increasing in recent years, however, it is desired to develop bioactive ceramics containing a carbonated apatite, a component of a bone in living body.

Because the carbonated apatites are lower in decomposition temperature than the hydroxyapatites, sintering is carried out at relatively low temperatures to provide carbonated apatite ceramics. JP 2000-72572 A discloses a molded implant produced by plastically working a sintered apatite body, and a method for producing the molded implant, which comprises the steps of sintering an apatite at 900°C or lower, filling the sintered apatite in a predetermined mold, and plastically working the sintered apatite at 300 to 780°C. In this method, because the sintering temperature is low, a carbonated or fluorinated apatite with low decomposition temperature can be used to produce the implant having high biocompatibility. However, this implant mainly comprises the apatite without other crystal phases, thereby having low mechanical strength.

The use of glass as a sintering aid is known to increase the mechanical strength of the bone restoration ceramic material composed of the apatite such as the carbonated apatite. In the sintering process, the glass is softened around main crystals of the apatite, and crystals are generated between the main crystals to be sintered, whereby the mechanical strength of the sintered apatite glass is increased. Conventionally, non-bioactive glasses are used as the sintering aid of the sintered hydroxyapatite body. However, because such non-bioactive glasses have high glass transition temperatures and/or crystallization temperatures, they cannot generate preferable crystals by sintering at temperatures lower than the decomposition temperatures of the carbonated apatites. Thus, the sintered carbonated apatite bodies using the non-bioactive glasses as sintering aids are not sufficient in the mechanical strength.

OBJECT OF THE INVENTION

Accordingly, an object of the present invention is to provide a bioactive glass low in a glass transition temperature and/or a crystallization temperature, and a sintered calcium phosphate glass that uses the bioactive glass to have high biocompatibility and mechanical strength.

SUMMARY OF THE INVENTION

As a result of intensive research in view of the above object, the inventors have found that a bioactive glass comprising 30 to 60 mol % of CaO, 40 to 70 mol % of SiO_2 and 20 mol % or less of Na_2O is low in a glass transition temperature and/or a crystallization temperature, and that a sintered calcium phosphate glass using the bioactive glass as a sintering aid is excellent in biocompatibility and mechanical strength. The present invention has been completed based on the findings.

Thus, the bioactive glass of the present invention has a composition substantially comprising 30 to 60 mol % of CaO, 40 to 70 mol % of SiO_2, and 20 mol % or less of Na_2O.

It is preferred that the bioactive glass of the present invention further comprises CaF_2 and/or B_2O_3. The bioactive glass preferably has a glass transition temperature of 790°C or lower. The difference between the glass transition temperature and the crystallization initiation temperature of the bioactive glass is preferably 80°C or more. The bioactive glass preferably forms a β-wollastonite crystal when crystallized.

In a preferred embodiment, the bioactive glass has a composition substantially comprising 30 to 60 mol % of CaO, 40 to 70 mol % of SiO_2, and at least one of Na_2O, CaF_2 and B_2O_3, Na_2O being 20 mol % or less, CaF_2 being 1 mol %, and B_2O_3 being 5 mol % or less. The bioactive glass is preferably substantially free from P_2O_5.

The sintered calcium phosphate glass of the present invention

comprises the bioactive glass of the present invention as a sintering aid.

A calcium phosphate contained in the sintered calcium phosphate glass of the present invention is preferably a hydroxyapatite, a carbonated apatite or tricalcium phosphate.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the result of differential thermal analysis of a CaO-SiO_2-based glass;

Fig. 2 is a schematic view showing the formation of β-wollastonite crystals in the process of sintering a CaO-SiO_2-based glass;

Figs. 3(a) to 3(d) are schematic, cross-sectional views showing the changes of particle boundaries in the process of sintering a green body composed of hydroxyapatite particles and CaO-SiO_2-based glass particles, wherein Fig. 3(a) shows the hydroxyapatite particles and the CaO-SiO_2-based glass particles at a temperature lower than a glass transition temperature, Fig. 3(b) shows the particles immediately after the temperature reaches the glass transition temperature, Fig. 3(c) shows densification by sintering with the formation of a grain boundary phase (glassy phase), and Fig. 3(d) shows the formation of β-wollastonite crystals after the temperature reaches a crystallization temperature;

Figs. 4(a) and 4(b) are graphs showing the results of X-ray structure analysis, wherein Fig. 4(a) shows the results of the bioactive glasses of Examples 1 to 6, and Fig. 4(b) shows the results of the bioactive glasses of Comparative Examples 1 to 5;

Fig. 5 is a graph showing the results of X-ray analysis of sintered calcium phosphate glass in Example 7;

Fig. 6 is a graph showing the results of X-ray analysis of sintered calcium phosphate glass in Example 8;

Fig. 7 is a graph showing the results of X-ray analysis of sintered hydroxyapatites in Comparative Example 6;

Fig. 8 is a photomicrograph with a magnification of 200 of HOS cells incubated on the carrier of Example 9 for one week; and

Fig. 9 is a photomicrograph with a magnification of 200 of HOS cells incubated on the carrier of Comparative Example 7 for one week.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[1] Bioactive glass

The bioactive glass of the present invention has a composition substantially comprising 30 to 60 mol % of CaO, 40 to 70 mol % of SiO_2, and 20 mol % or less of Na_2O, and more preferably has a composition substantially comprising 40 to 50 mol % of CaO, 40 to 50 mol % of SiO_2, and 20 mol % or less of Na_2O. The glass with such a composition has bioactivity preferable for use as a bioactive material, and has mechanical strength, sinterability, etc. preferable for use as a sintering aid in a sintered calcium phosphate glass.

The bioactive glass comprising CaO releases calcium ions in a living body, thereby showing bioactivity. The bioactive glass, which has lost part of the calcium ions by elution, forms a silica gel layer mainly composed of silicon oxide. The silica gel layer forms the basis of nucleation of calcium phosphate crystals, whereby the bioactive glass can strongly connect to cortical bones.

The bioactive glass of the present invention comprises CaO and SiO_2 as main components with approximately equal molar ratios. Thus, the composition of the bioactive glass is substantially the same as that of the β-wollastonite, whereby the bioactive glass easily generates β-wollastonite crystals at a crystallization temperature. The crystal

generated at the crystallization temperature is preferably a β-wollastonite crystal having a needle-like structure, because the mechanical strength of the sintered calcium phosphate glass is more increased by such a β-wollastonite crystal as compared with other crystals. In the case of adding a large amount of P_2O_5 to improve biocompatibility by conventional methods, however, the formation of the β-wollastonite crystal is often prevented at a crystallization temperature.

The bioactive glass of the present invention has improved biocompatibility with increased CaO content, needing no P_2O_5. In addition, because the glass transition temperature and/or the crystallization temperature of the bioactive glass are often increased by P_2O_5, the bioactive glass of the present invention is thus substantially free from P_2O_5. The bioactive glass of the present invention containing substantially no P_2O_5 easily generates the β-wollastonite crystal.

In the bioactive glass of the present invention, the total molar ratio of CaO and SiO_2 is preferably 90 mol % or more, more preferably 95 mol % or more.

Crystals of tricalcium phosphate $Ca_3(PO_4)_2$ may be generated at the crystallization temperature. Tricalcium phosphate is similar in physical properties, solubility and biocompatibility, to hydroxyapatites. Further, the crystal of tricalcium phosphate can improve the biocompatibility of the sintered calcium phosphate glass.

The sinterability is improved in a case where the sintering aid of the bioactive glass has (1) a low glass transition temperature Tg, (2) a crystallization initiation temperature Tc_0 remarkably lower than a decomposition temperature of calcium phosphate, and (3) a large difference ΔT between the glass transition temperature and the

crystallization initiation temperature Tc_0. In the present invention, the term "crystallization initiation temperature" means a temperature at which the bioactive glass begins to generate a crystal such as the β-wollastonite crystal. Specifically, the crystallization initiation temperature is defined as a temperature of intersection of a base line and a bottom of an exothermic peak in a differential thermal analysis curve. The term "crystallization temperature" means a temperature at which the crystal is generated, with a definition as a temperature of an exothermic peak in a differential thermal analysis curve.

To evaluate the effects of Na_2O, etc. in a system of CaO, SiO_2 and Na_2O on the glass transition temperature, etc., a bioactive glass composed of 50 mol % of CaO and 50 mol % of SiO_2 is hereinafter used as a control.

The graph of Fig. 1 shows the exothermic and endothermic changes with temperature in the differential thermal analysis of a bioactive glass composed of 50 mol % of CaO and 50 mol % of SiO_2 from 100°C to 1100°C. The bioactive glass generates heat in a temperature range where the curve is above the line L, and absorbs heat in a temperature range where the curve is below the line L. A tangential line *a* at the inflection point of the curve at the beginning of heat absorption, an approximate line *b* (base line), and a tangential line *c* at the inflection point of the curve in the rising of an exothermic peak are given to the differential thermal analysis curve in the temperature range showing the endothermic changes. The glass transition temperature Tg is obtained from the intersection of the tangential line *a* and the approximate line *b*, and the crystallization initiation temperature Tc_0 is obtained from the intersection of the approximate line *b* and the tangential line *c*. In Fig. 1, each of Tc_1 and Tc_2 represents the crystallization temperature, and ΔT represents the difference of the glass transition temperature Tg and the crystallization

initiation temperature Tc_0. The bioactive glass shows a softening behavior in a temperature region between the glass transition temperature Tg and the crystallization initiation temperature Tc_0.

The bioactive glass with a low glass transition temperature Tg can be used as a sintering aid for the carbonated apatite, etc. having a low decomposition temperature. To easily sinter the bioactive glass at a temperature lower than the decomposition temperature of calcium phosphate and higher than the crystallization initiation temperature Tc_0, the crystallization initiation temperature Tc_0 is preferably lower than the decomposition temperature with a difference of approximately 400°C or more. The glass transition temperature Tg is preferably 790°C or lower, more preferably 770°C or lower. Further, the bioactive glass of the present invention preferably has a large difference ΔT between the glass transition temperature and the crystallization initiation temperature. When the difference ΔT is large, dense crystals are easily obtained without needing precise control of the sintering temperature. The difference ΔT of the bioactive glass is preferably 80°C or more, more preferably 90°C or more.

The glass transition temperature Tg of the bioactive glass may be lowered by adding Na_2O. However, an excess amount of Na_2O often inhibits the formation of the β-wollastonite crystal. Thus, the amount of Na_2O is preferably 10 mol % or less, more preferably 5 mol % or less, particularly preferably 1 mol % or less. The lower limit of the amount of Na_2O is preferably 0.1 mol %. When the amount of Na_2O added is less than 0.1 mol %, the effects of adding Na_2O are substantially not obtained.

The addition of CaF_2 to the bioactive glass can lower its glass transition temperature Tg and increase the difference ΔT. With CaF_2 added, the glass transition temperature Tg and the crystallization initiation

temperature Tc_0 are both lowered, and the reduction of the crystallization initiation temperature Tc_0 is smaller than that of the glass transition temperature Tg. Thus, the glass transition temperature Tg is lowered, and the difference ΔT is increased. The amount of CaF_2 added is preferably 1 mol % or less, more preferably 0.5 mol % or less.

B_2O_3 may be added to the bioactive glass. The addition of a small amount of B_2O_3 can lower its glass transition temperature Tg and crystallization initiation temperature Tc_0 and increase the difference ΔT like the addition of CaF_2. The amount of B_2O_3 added is preferably 5 mol % or less, more preferably 1 mol % or less.

At least one of Na_2O, CaF_2 and B_2O_3 should be contained in the bioactive glass of the present invention. It is preferable that Na_2O, CaF_2 and B_2O_3 are added to the bioactive glass in combination. The bioactive glass with the preferred glass transition temperature Tg and the preferred difference ΔT can be obtained by appropriately combining Na_2O, CaF_2 and B_2O_3. The total amount of Na_2O, CaF_2 and B_2O_3 is preferably 5 mol % or less, more preferably 2 mol % or less. The lower limit of the total amount of Na_2O, CaF_2 and B_2O_3 is preferably 0.1 mol%.

An inorganic compound such as K_2O, Li_2O, TiO_2, Al_2O_3, MgO and ZrO_2 may be added to the bioactive glass. It is preferable to use an inorganic compound that does not increase the glass transition temperature Tg and does not inhibit the formation of the β-wollastonite crystal.

There are no particular restrictions in a method for producing the bioactive glass of the present invention. The bioactive glass may be produced by a method described in JP 60-239341 A, etc. Specifically, powders of materials (CaO, SiO_2, Na_2O, CaF_2, B_2O_3, etc.) with a desired composition are put in a platinum crucible and heated at 1,200°C to 1,600°C for approximately 3 hours to obtain a molten glass. The molten

glass is molded and annealed to produce the bioactive glass. Though not particularly restrictive, the shape of the bioactive glass may be selected in a shape of an ingot, a sphere, beads, particles, granules, etc. depending on the purposes. When the bioactive glass is used as a starting material for the sintered calcium phosphate glass of the present invention that will be described below, the diameter of the bioactive glass may be controlled by pulverization or classification.

[2] Sintered calcium phosphate glass

(a) Composition of sintered calcium phosphate glass

A calcium phosphate contained in the sintered calcium phosphate glass of the present invention is preferably a hydroxyapatite, a carbonated apatite or tricalcium phosphate.

When the hydroxyapatite is heated, it is gradually deprived of hydroxyl groups at around 1,000°C or higher, causing decomposition at around 1,300°C or higher. Thus, in the case of using the hydroxyapatite for the sintered calcium phosphate glass, the sintering process is preferably carried out at a temperature lower than 1,000°C.

The biocompatibility of the sintered calcium phosphate glass may be further increased by using the carbonated apatite. The carbonate moieties of the carbonated apatite are eliminated at a temperature of around 900°C or higher, which is lower than the elimination temperature of the hydroxyl groups of the hydroxyapatite. Thus, in the case of using the carbonated apatite for the sintered calcium phosphate glass, the sintering process is preferably carried out at a temperature lower than 900°C.

The sintered calcium phosphate glass of the present invention comprises the bioactive glass of the present invention as a sintering aid. The bioactive glass preferably generates the β-wollastonite crystals at the

crystallization temperature as shown in Fig. 2. The percentage of the generated β-wollastonite crystals to the bioactive glass is preferably 10 to 100% by mass.

(b) Method for producing sintered calcium phosphate glass

The sintered calcium phosphate glass of the present invention may be produced by a common sintering method.

The average particle diameter of the calcium phosphate particles is preferably 1 to 100 μm, more preferably 10 to 20 μm. The calcium phosphate particles with such an average particle diameter may be prepared by a spray granulation method. Thus, the calcium phosphate particles are agglomerates of fine calcium phosphate crystals (primary particles). The calcium phosphate crystal is preferably in the form of nano-particles having diameters of 1 μm or less, more preferably nano-particles having diameters of 10 to 500 nm.

The pulverized particles of the bioactive glass of the present invention may be added to the calcium phosphate particles. The average particle diameter of the bioactive glass particles is preferably 0.1 to 10 μm, more preferably 5 μm or less. The percentage of the bioactive glass to the calcium phosphate particles is preferably 0.5 to 10% by mass, more preferably 1 to 5% by mass.

The calcium phosphate particles and the bioactive glass particles may be wet-blended with alumina balls and a solvent such as isopropyl alcohol, ethanol, etc., and dried to obtain a mixture for sintering. The drying time is preferably 0.5 to 5 hours, more preferably 2 to 5 hours. The mixture is preferably put in a stainless steel die, etc. and press-molded and then cold-isostatic-pressed.

A green body thus obtained is sintered. The sintering

temperature of the green body is preferably 700 to 1300°C, more preferably 700 to 900°C. The sintering time is preferably 0.5 to 10 hours, more preferably 2 to 5 hours. The sintering process is described with reference to the schematic views of Fig. 3(a) to 3(d). As shown in Fig. 3(a), the calcium phosphate particles and the glass particles are uniformly distributed in the green body. When the green body is heated at the glass transition temperature or higher, the glass particles are softened as shown in Fig. 3(b). When the green body is further heated, the softened glass particles penetrate into pores between the calcium phosphate particles to cause densification, thereby forming grain boundary phases (glassy phases) as shown in Fig. 3(c).

As shown in Fig. 3(d), when the sintering process proceeds and the green body is heated at a temperature at which at least part of the glass components forms crystals, crystals are generated in the grain boundary phase to form crystal phases. Because the sintering temperature is lower than the melting temperature and the decomposition temperature of the calcium phosphate throughout the sintering process, the calcium phosphate particles are hardly decomposed or dissolved in the glass. Thus, the crystals such as the β-wollastonite crystals of certain glass components are generated between the calcium phosphate crystals, to provide the sintered, dense calcium phosphate glass. The heating rate is preferably uniform, and preferred heating rate is approximately 10°C/min. The sintering temperature is preferably maintained between the glass transition temperature and the crystallization temperature for 1 to 5 hours. The sintered calcium phosphate glass is preferably cooled in a furnace.

The present invention will be explained in more detail with reference to Examples below without intention of restricting the scope of the present invention.

Example 1

49.5 mol % of CaO powder, 49.5 mol % of SiO_2 powder, and 1 mol % of Na_2O powder were mixed and melted at 1500°C for 2 hours, to produce a bioactive glass ingot having a uniform composition.

Examples 2 to 6

Material powders were melted at 1500°C for 2 hours, to produce bioactive glass ingots having uniform compositions shown in Table 1.

Table 1

Bioactive Glass	Composition (mol %)				
	CaO	SiO_2	Na_2O	CaF_2	B_2O_3
Example 1	49.5	49.5	1.0	-	-
Example 2	47.5	47.5	5.0	-	-
Example 3	40.0	50.0	10.0	-	-
Example 4	49.5	50.0	-	0.5	-
Example 5	49.0	49.5	1.0	0.5	-
Example 6	49.5	49.0	1.0	0.5	1.0

Comparative Examples 1 to 5

Material powders were melted at 1500°C for 2 hours, to produce bioactive glass ingots having uniform compositions shown in Table 2.

Table 2

Bioactive Glass	Composition (mol %)		
	CaO	SiO_2	P_2O_5
Comparative Example 1	50.0	50.0	-
Comparative Example 2	49.0	51.0	-
Comparative Example 3	40.0	60.0	-
Comparative Example 4	47.5	47.5	5.0
Comparative Example 5	60.0	30.0	10.0

Each bioactive glass of Examples 1 to 6 and Comparative Examples 1 to 5 was subjected to differential thermal analysis, to obtain the glass transition temperature Tg, the crystallization initiation temperature Tc_0, the crystallization temperature Tc, and the difference ΔT between the glass transition temperature and the crystallization initiation temperature.

As shown in Table 3, each bioactive glass of Examples 1 to 6 had a lower glass transition temperature Tg as compared with the bioactive glasses of Comparative Examples free of Na_2O, etc. Each bioactive glass of Examples 4 to 6 containing CaF_2 had a relatively large difference ΔT.

Table 3

Bioactive Glass	Glass Transition Temperature Tg (°C)	Crystallization Initiation Temperature Tc_0 (°C)	Crystallization Temperature Tc (°C)	Difference ΔT between Tg and Tc_0 (°C)
Example 1	774.4	862.5	882.6	88.1
Example 2	717.4	859.0	829.3	141.6
Example 3	662.9	726.0	753.0 814.3 918.2	63.1
Example 4	780.4	862.6	883.8	82.2
Example 5	763.1	859.0	874.9	95.9
Example 6	746.4	837.4	851.6 869.4	91.0
Comparative Example 1	792.9	861.8	880.6 914.7	68.8
Comparative Example 2	789.6	866.7	886.2	77.1
Comparative Example 3	780.8	882.2	911.9	101.4
Comparative Example 4	789.1	896.1	944.9	107.0
Comparative Example 5	807.2	873.4	885.9	66.2

Each bioactive glass of Examples 1 to 6 and Comparative Examples 1 to 5 was heated at the crystallization temperature or higher, and the generated crystals were analyzed by X-ray structure analysis. The results of X-ray analysis of Examples 1 to 6 are shown in the graphs of Fig. 4(a), and the results of Comparative Examples 1 to 5 are shown in the graphs of Fig. 4(b).

As shown in Table 4, the β-wollastonite crystals were mainly generated in the bioactive glasses of Examples 1, 2, and 4 to 6, and

Comparative Examples 1 to 3, which contained approximately the same molar amount of CaO and SiO_2. On the other hand, the β-wollastonite crystals were hardly generated in the bioactive glasses of Comparative Examples 4 and 5 containing P_2O_5.

Table 4

Bioactive Glass	Crystallization Temperature Tc (°C)	Crystal System
Example 1	882.6	β-wollastonite » Na_2CaSiO_4[(1)]
Example 2	829.3	β-wollastonite > Na_2CaSiO_4, $Na_2Ca_2SiO_7$[(2)]
Example 3	753.0 814.3 918.2	$Na_2CaSi_3O_8$ » Na_2CaSiO_4 $Na_2CaSi_3O_8$ » Na_2CaSiO_4 $Na_2CaSi_3O_8$ » Na_2CaSiO_4
Example 4	883.8	β-wollastonite
Example 5	874.9	β-wollastonite » Na_2CaSiO_4
Example 6	851.6 869.4	Na_2CaSiO_4 > β-wollastonite β-wollastonite > Na_2CaSiO_4
Comparative Example 1	880.6 914.7	β-wollastonite β-wollastonite
Comparative Example 2	886.2	β-wollastonite
Comparative Example 3	911.9	β-wollastonite
Comparative Example 4	944.9	α-wollastonite > β-wollastonite
Comparative Example 5	885.9	Ca_2SiO_4 > Ca $(PO_3)_2$

Notes: (1) "»" means that the crystal on the left side was generated in an extremely larger amount.

(2) ">" means that the crystal on the left side was generated in a larger amount.

Example 7

The bioactive glass ingot of Example 1 was pulverized into particles with an average particle diameter of 10 μm, and 5% by mass thereof was added to 100% by mass of agglomerated particles (average diameter: 15 μm) of hydroxyapatite nano-particles available from Pentax Corporation. The resultant mixture was wet-blended using isopropyl alcohol and alumina balls, and dried to obtain powder for sintering. 0.2 g of the powder was placed in a stainless steel die, and press-molded and cold-isostatic-pressed (CIP), and finished to produce a disc-shaped green body having a diameter of 10 mm and thickness of 2 mm. The green body was sintered at 900°C for 3 hours and cooled in a furnace to produce a sintered body of the hydroxyapatite glass. The heating rate in the sintering was 10°C/min. Further, three sintered bodies of the hydroxyapatite glass were produced in the same manner except for changing the sintering temperature to 1,000°C, 1100°C or 1200°C, respectively. The sintered bodies and the unsintered green body were subjected to X-ray analysis. The results of the X-ray analysis are shown in the graph of Fig. 5.

Example 8

Four sintered bodies of the same hydroxyapatite glass were produced by sintering at different temperatures in the same manner as in Example 7 except for using the bioactive glass of Example 5. The sintered bodies and the unsintered green body were subjected to X-ray analysis. The results of the X-ray analysis are shown in the graph of Fig. 6.

Comparative Example 6

The hydroxyapatite green bodies in Examples 7 and 8 were sintered at 900°C, 1,000°C, 1,100°C or 1,200°C, respectively, for 3 hours. The resultant sintered bodies and the unsintered green body were subjected to X-ray analysis. The results of the X-ray analysis are shown in the graph of Fig. 7.

In the case of Comparative Example 6, only peaks of the hydroxyapatite were detected irrespective of the sintering temperature. In the case of the sintered bodies of Examples 7 and 8, which contained the bioactive glasses, those sintered at 1,000°C or higher showed peaks of the β-wollastonite, and those sintered at 1,100°C or higher further showed peaks of β-tricalcium phosphate. The β-wollastonite phase is preferable for reinforcing the grain boundaries, and the β-tricalcium phosphate phase is preferable for enhancing bioactivity.

Example 9

The bioactive glass produced in Example 5 were examined with respect to cell attachment, cell proliferation and alkaline phosphotase activity as follows: A test piece (5 mm × 5 mm × 2 mm) of the bioactive glass of Example 5 was subjected to high-pressure steam sterilization, and placed in a 24-well multiplate for cell culture (available from Sumitomo Bakelite Co., Ltd., diameter: 16.3 mm, base area: 1.8 cm^3). 1.0×10^4 HOS cells derived from human osteosarcoma (ATCC No. CRL-1543) were seeded in each plate, and 1 ml of D-MEM 10% FBS (available from GIBCO-BRL) was added to the plate. The cells were incubated at 37°C for 60 minutes or 7 days in air with a 5-% CO_2 content. The culture

medium was exchanged on the fourth day of the 7-days incubation.

Comparative Example 7

HOS cells were incubated in the same manner as in Example 9 except for using a test piece (diameter: 6 mm × 2 mm) of the sintered hydroxyapatite body of Comparative Example 6 (sintering temperature: 1,000°C) instead of the bioactive glass as a carrier. The cell attachment, cell proliferation and alkaline phosphotase activity of the sintered hydroxyapatite body were examined.

The incubated cells were fixed by a 10-%, neutral, buffered formalin solution, stained by methylene blue, and observed by an optical microscope and an electron microscope. To evaluate cell differentiation, the incubated cells were homogenized and the alkaline phosphotase activity was measured by Alkalipha K-test Wako (available from Wako Pure Chemical Industries, Ltd.).

Adhesion of the cells to each carrier used in Example 9 and Comparative Example 7 was observed after the 60-minute incubation. In the case of the carrier according to Example 9, the cells were proliferated on the bioactive glass, and were nearly in a confluent state on the fourth day of the incubation. After the 7-days incubation, the cells were proliferated on each carrier of Example 9 and Comparative Example 7 into a confluent state. Photomicrographs (a magnification of 200) of the HOS cells incubated for a week are shown in Figs. 8 and 9. Fig. 8 shows the HOS cells incubated on the carrier of Example 9, and Fig. 9 shows the HOS cells incubated on the carrier of Comparative Example 7. Further, numbers of the cells, attached to the bioactive glass and the sintered hydroxyapatite body after the incubation of 60 minutes and 7 days, are shown in Table 5. The carrier of Example 9 provided excellent cell

proliferation, as well as the carrier of Comparative Example 7.

Table 5

Carrier	Number of Attached Cells	
	Incubation Period	
	60 minutes	7 days
Example 9	$6.8 \times 10^3/cm^2$	$1.8 \times 10^5/cm^2$
Comparative Example 7	$6.0 \times 10^3/cm^2$	$2.0 \times 10^5/cm^2$

The alkaline phosphotase activities after the incubation of 7 days are shown in Table 6. The carrier of Example 9 was higher in the alkaline phosphotase activity than the carrier of Comparative Example 7. This result indicates that the bioactive glass affects the cell differentiation.

Table 6

Carrier	Alkaline Phosphotase Activity per 1 cm^2 (unit: K-A)
Example 9	2.4
Comparative Example 7	1.1

As described in detail above, the bioactive glass of the present invention has a composition substantially comprising 30 to 60 mol % of CaO, 40 to 70 mol % of SiO_2 and 20 mol % or less of Na_2O. By containing CaO and SiO_2 as main components, the bioactive glass easily generates the β-wollastonite crystal at the crystallization temperature, resulting in excellent mechanical strength. By containing Na_2O, the

bioactive glass has a low glass transition temperature and/or crystallization temperature. Further, when the bioactive glass of the present invention contains CaF_2 and/or B_2O_3, the difference between the glass transition temperature and the crystallization temperature is increased. The sintered calcium phosphate glass of the present invention comprises the bioactive glass as a sintering aid, thereby exhibiting high biocompatibility and excellent mechanical strength and sinterability. The present disclosure relates to subject matter contained in Japanese Patent Application No. 2002-206319 (filed on July 15, 2002) which is expressly incorporated herein by reference in its entirety.